Docket No. 56876

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: K. OSTHER et al.

EXPRESS MAIL LABEL NO. EL 885010382 US

FOR: IN VITRO REPAIR OF BONEAND/OR CARTILAGE DEFECTS

Honorable Commissioner of Patents and Trademarks
Washington, DC 20231

Dear Sir:

PRELIMINARY AMENDMENT

Applicants file herewith the above-identified application. Please amend the application as follows.

IN THE CLAIMS

Please cancel claims 1-28 without prejudice.

Please add the following new claims.

29. A cartilage membrane comprising at least one surface part carrying a composition comprising at least one stimulation molecule, which induces a signal transduction in chondroblast/chondrocytes and which is selected from the group consisting of collagen proteins, proteoglycans, and non-collageneous proteins.

30. A cartilage membrane according to claim 29 wherein the collagen protein is collagen types II, VI, IX, or XI, the proteoglycan is aggregans, decorin, fibromodulin or biglycan, and the non-collageneous protein is cryoprecipitate, fibronectln, vitronectin, fibronogen, fibrillin, kistrin, echistatitn von Willebrand factor, tenascin or anchorin CII.

31. A cartilage membrane according to claim 29, which is a non-immunogenic, non-toxic, biodegradable membrane.

32. A cartilage membrane according to claim 29, wherein the membrane material is porous or substantially porous.

33. A cartilage membrane according to claim 32, wherein the membrane is a natural or synthetic collagen type I membrane or part thereof.

34. An interface membrane with a first surface part and a second surface part both carrying a composition comprising at least one stimulation molecule which induces a signal transduction in chondroblast/chondrocytes and in osteoblasts/osteocytes and which is selected from the group consisting of collagen proteins, proteoglycans, and non-collageneous proteins.

35. An interface membrane according to claim 34 wherein the collagen protein is collagen types II, VI, IX, or XI, the proteoglycan is aggregans, decorin, fibromodulin or biglycan, and the non-collageneous protein is cryoprecipitate, fibronectln, vitronectin, fibronogen, fibrillin, kistrin, echistatitn von Willebrand factor, tenascin or anchorin CII.

36. An interface membrane according to claim 34, which is a non-immunogenic, non-toxic, biodegradable membrane.

37. An interface membrane according to claims 34, wherein the membrane material is porous or substantially porous.

38. An interface membrane according to claim 37, wherein the membrane is a natural or synthetic collagen type I membrane or part thereof.

39. A membrane according to claim 29 or 34, wherein the stimulation molecule comprises at least one RGD motif.

40. A membrane according to claim 39, wherein the stimulation molecule is a natural or synthetic protein or peptide or a fusion or a mixture thereof.

41. A membrane according to claim 40, wherein the stimulation molecule is selected from the group consisting of collagen type II and fibronectin.

42. A membrane according to claim 41, wherein the stimulation molecule is attached to a support.

43. A method for in vivo repair of cartilage defects in joints in a mammal, comprising

i) applying, over a cartilage free cavity of a joint, a cartilage membrane with a first surface part of which facing the cartilage free cavity, the first surface part of the cartilage membrane carrying a composition comprising at least one stimulation molecule which can induce a signal transduction in chondroblast/chondrocytes,

ii) introducing, in the cartilage free cavity between the cartilage membrane, the cartilage and the interface, a chondroblast/chondrocyte suspension, and

iii) joining a portion part of the first surface part of the cartilage membrane to the surrounding articular surface so as to sealing entrap the chondroblast/chondrocyte suspension in the cartilage free cavity using a scaling portion, thereby allowing the chondroblast/chondrocyte suspension to produce and secrete matrix components characteristic for hyaline.

44. A method for in vivo repair of bone and cartilage defects in joints in a mammal, comprising:

i) applying, over a bone free cavity and under a cartilage free cavity of a joint, an interface membrane with a first surface part facing the bone free cavity, the interface membrane first surface part carrying a composition comprising at least one stimulation molecule which induces a signal transduction in osteoblast/osteocyte, and the second surface part carrying a composition comprising at least one stimulation molecule which can induce a signal transduction in chondroblast/chondrocytes,

ii) introducing, in the interstice between the interface membrane first surface part and the bone, an osteoblast/osteocyte suspension,

iii) joining a portion part of the first surface part of the interface membrane to the surrounding interface surface so as to sealingly entrap the osteoblast/osteocyte suspension in the bone free cavity using a sealing portion, thereby allowing the osteoblast/osteocyte suspension to produce and secrete matrix components characteristic for bone tissue,

iv) applying, over the cartilage free cavity, a cartilage membrane with a first surface part facing the second surface part of the interface membrane, the first surface part of the cartilage membrane carries a composition comprising at least one stimulation molecule which can induce a signal transduction in chondroblast/chondrocytes resulting in the chondroblast/chondrocytes producing and secreting matrix components which form hyalin cartilage,

v) introducing, in the cartilage free cavity between the interface membrane, the cartilage membrane and the cartilage, a chondroblast/chondrocyte suspension,

vi) joining a portion part of the cartilage membrane to the surrounding articular surface so as to sealingly entrap the chondroblast/chondrocyte suspension in the cartilage free cavity using a sealing portion, thereby allowing the chondroblast/chondrocyte suspension to produce and secrete matrix components which form hyalin.

45. A method for in vivo repair of bone and cartilage defects in joints in a mammal using arthroscopy, comprising:

i) treating an interface membrane with a first sealing portion component, applying, over a bone free cavity and under a cartilage free cavity of a joint, an interface membrane with a first surface part facing the bone free cavity, the interface membrane first surface part carrying a composition comprising at least one stimulation molecule which induces a signal transduction in osteoblast/osteocyte, and the second surface part, which carries a composition comprising at least one stimulation molecule which can induce a signal transduction in chondroblast/chondrocytes,

ii) introducing, in the interstice between the interface membrane first surface part and the bone, an osteoblast/osteocyte suspension,

iii) joining a portion part of the first surface part of the interface membrane to the surrounding interface surface so as to sealingly entrap the osteoblast/osteocyte suspension in the bone free cavity using a second sealing portion component, thereby allowing the osteoblast/osteocyte suspension to produce and secrete components characteristic for bone tissue,

iv) introducing, in the cartilage free cavity between the interface membrane, and the articular surface, a chondroblast/chondrocyte suspension, thereby allowing the

chondroblast/chondrocyte suspension to produce and secrete components characteristic for hyaline.

46. A method according to claim 43, 44 or 45 wherein the cartilage membrane comprises at least one surface part carrying a composition comprising at least one stimulation molecule, which induces a signal transduction in chondroblast/chondrocytes and which is selected from the group consisting of collagen proteins proteoglycans, and non-collageneous proteins.

47. A method according to claim 46 wherein the collagen protein is collagen types II, VI, IX, or XI, the proteoglycan is aggregans, decorin, fibromodulin or biglycan, and the non-collageneous protein is cryoprecipitate, fibronectln, vitronectin, fibronogen, fibrillin, kistrin, echistatitn von Willebrand factor, tenascin or anchorin CII.

48. A method according to any of claims 43, 44 or 45, wherein the chondroblast/chondrocyte suspension is a suspension of autologous chondroblast/chrondrocytes.

49. A method according any of claims 43, 44 or 45, wherein the osteoblast/osteocyte suspension is a suspension of autologous osteoblast/osteocyte.

50. A method according to any of the claims 43, 44 or 45 wherein the mammal has cartilage defects or bone and cartilage defects.

51. A method according to any one of claims 43, 44 or 45 wherein the mammal is suffering from chondral lesions or osteochondreal lesions, osteochondritis dissecans, chondromalacia or osteoathritis.

52. A kit for cartilage repair comprising a cartilage membrane that comprises at least one surface part carrying a composition comprising at least one stimulation molecule, which induces a signal transduction in

chondroblast/chondrocytes and which is selected from the group consisting of collagen proteins proteoglycans, and non-collageneous proteins.

53. A method for preparation of chondroblast/chondrocyte or osteocytelosteoblast suspensions comprising

i) harvesting mesenchymal and/or mesenchymal precursor cells from a source such as bone marrow, perichondrium, periosteum, blood, blood vessels or muscle,

ii) adding the harvested cells to a cell culture flask comprising at least one growth medium,

iii) growing the harvested cells until colony forming units with a cell number size in the ranging order of 10-20,000 cells/clone are formed with fibroblastic phenotype (CFU-f),

iv) transferring the CFU-f cells into a new cell culture flask comprising at least one selection medium for differentiation of the CFU-f's into chondroblast/chondrocytes, osteocytes/osteoblasts or myoblasts/myotubes, and

v) harvesting of the differentiated cells.

54. A method according to claim 53, wherein the suspensions are used for the treatment of cartilage and/or bone and cartilage defects in mammals.

55. A method according to claim 53, wherein the selection medium comprises components more specific for selection than for growth.

56. A cell cultivation method for preparation of chondroblast/chondrocyte without enzymatic treatment comprising harvesting cartilage explants from a mammal;

i) adding the harvested cartilage explants to a culturing flask comprising at least one growth medium;

ii) growing the cartilage explants in the medium to obtain a chondroblast/chondrocyte a mono-layer,

iii) propagation of the cartilage explains until several mono-layers and a high cell number are obtained and

iv) transferring the mono-layer culture into an autologous growth medium.

57. A method according to claim 56, wherein the cartilage explants are used for the treatment of cartilage and/or bone and cartilage defects in mammals.

REMARKS

To reduce initial filing fees and place the claims in U.S. format, claims 1-29 have been cancelled without prejudice, and claims 29-57 have been added. No new matter has been added.

Early consideration and allowance of the application are earnestly solicited.

Respectfully submitted,

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